Ref. No.: CML-STY-0049A/01

# STUDY PROTOCOL

## CCQM-K109 and CCQM-P148

## High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum

### Background

The OAWG has agreed on a list of Track A key comparisons to assess the core competencies of National Metrology Institutes/Designated Institutes (NMIs/DIs) for the delivery of measurement services to their customers. One of the Track A comparisons discussed and agreed upon under the matrix category was “Polar Organic in Biological Matrix”. In the OAWG meeting in November 2013 in Pretoria, South Africa, the meeting discussed the possible analytes and biological materials in this comparison which could best cover current and future CMCs. The Health Sciences Authority (HSA), Singapore suggested urea and uric acid in human serum as a possible comparison for this category, and would present a proposal at the meeting in April 2014 in BIPM. At the April 2014 OAWG meeting, a proposal was presented by HSA. After considering the services offered by the NMIs/DIs and that this being the first Track A comparison for biological materials, the OAWG agreed on the HSA’s proposal on urea and uric acid in human serum as an appropriate comparison for this matrix category. A key comparison and a parallel pilot study will be organised.

### Objectives

The comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of high polarity organic compounds in a biological matrix. As a model system for this comparison, two polar clinical biomarkers: urea and uric acid, in human serum are chosen.

## Preparation of the Comparison Materials

The comparison materials are frozen human sera. An experienced commercial human blood products supplier (Solomon Park Research Laboratories, Kirkland, WA, USA) was engaged by the Health Sciences Authority (HSA) to prepare the materials. Two pools of human serum materials with two different concentration levels of urea and uric acid were prepared, and pre-packed in 260 vials containing 1 mL of serum each.

The homogeneity of the comparison materials was assessed by gas chromatography-isotope dilution mass spectrometry (GC-IDMS). A sample size of 0.10 g was used in the assessment of homogeneity for both urea and uric acid. Eleven bottles were randomly and stratifically selected, and two subsamples were taken from each bottle. Using ANOVA at 95 % level of confidence, both materials were found to be sufficiently homogeneous. The relative standard uncertainties[1] of inhomogeneity were found to be below 0.04 % and 0.18 % for urea and uric acid (for both concentration levels), respectively.

The stability of the comparison materials at – 70 °C was assessed using the GC-IDMS and liquid chromatography-isotope dilution mass spectrometry (LC-IDMS) for urea and uric acid, respectively. The same sample size as described in the homogeneity testing was also used. The testing was carried out on four occasions over a period of about 230 days using classical design. For each occasion of the stability testing, two bottles were randomly selected, and two subsamples were taken from each bottle. Using Student's *t*-test at 95 % level of confidence, no significant instability of the comparison materials was observed. The relative standard uncertainties of instability were estimated to be below 0.21 % and 0.46 % for urea and uric acid (for both concentration levels), respectively.

A study on the effect of different equilibration times (2 h to 29.5 h) on the measurement results of urea and uric acid for serum materials containing a high and low level of lipid was carried out. The results showed that equilibration times do not have a significant effect on the measurement results of urea and uric acid for serum materials with either high or low level of lipid.

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[1] As the between-bottle mean squares were found to be smaller than the within-bottle mean squares, the uncertainties for inhomogeneity were estimated using the following equation:

$$\sqrt{\frac{MS_w}{n}}\sqrt[4]{\frac{2}{v_{MS_w}}}$$

where $MS_w$ is the within-bottle mean square, $n$ is the number of observations, $v_{MSw}$ is the number of degrees of freedom of $MS_w$.

## The Measurands

The mass fractions of urea and uric acid in the study materials are in the range of 100 to 2,000 and 10 to 165 mg/kg, respectively. One of the concentration levels is within the normal biological range while the other is higher than normal range. The concentration levels are within the range of existing CMC claims in the BIPM KCDB.

## Registration

Interested institutes should complete the Registration Form and return to HSA before the deadline and an email will be sent to confirm the registration. The institutes may choose to register for one or both the measurands. Potential DIs that are nominated by their respective NMIs are welcome to participate in the pilot study.

## Instructions for Participating Institutes

The materials used for this comparison were tested non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV) by the supplier before distribution. However, the materials should be handled as biohazards materials capable of transmitting infectious diseases.

The materials will be transported using dry ice. Upon receipt, the materials should be immediately stored at a temperature below – 60 °C before measurement. The materials should be used immediately after they are thawed as measurements on vials, which have been previously thawed and opened, have not been conducted.

Each participating NMI/DI will receive three vials of serum sample for each concentration level and measurand that it registers for, i.e. the NMI/DI will receive a total of 12 vials if it registers for both urea and uric acid. The participating NMIs/DIs may use one of the three vials as a practice sample and should report the results for the remaining two vials. At least two subsamples should be taken from each vial. The participating NMIs/DIs may decide on the number of times that each subsample is to be measured. Before sampling, the material should be allowed to thaw and warm to room temperature (18 – 25 °C), and homogenised by gentle swirling and inversing the vial several times. The subsamples taken from the same vial should be measured on the same day. The recommended minimum subsample size is 0.10 g.

The participating NMIs/DIs should use their own methods for the determination. Metrologically traceable certified reference materials (CRMs) should be used as calibration standards. CRMs of urea and uric acid are available from the National Institute of Science and Technology (NIST), the National Metrology Institute of Japan (NMIJ), and the National Institute of Metrology (NIM), China. Other sources of reference materials may be used, provided that they are purity assessed adequately to demonstrate the metrological traceability. It is recommended to use matrix CRMs as quality controls. CRMs for urea and uric acid in human serum are available from the Korea Research Institute of Standards and Science (KRISS), NIST, HSA and NIM, China. Please refer to the Appendix[2] for more information.

## Internal Standards

Isotopic labelled urea is available from Cambridge Isotopes Laboratories, and isotopic labelled uric acid is available from Sigma Aldrich and Cambridge Isotopes Laboratories.

## Reporting of Results

A Report of Results Form will be provided to the participating NMIs/DIs for completion. The participating NMIs/DIs are expected to report their results based on at least four subsamples (two subsamples from each vial) for each level. The results should be reported in the unit of mg/kg, and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure, the calibration standard, the internal standard, the quality control material, the calculation of the results, and the estimation of measurement uncertainty should be included. The completed form should be sent to HSA on or before the scheduled deadline. The submitted results will be considered as final.

## Evaluation of Results

Results of all participating NMIs/DIs will be evaluated against the key comparison reference value (KCRV). The KCRV and associated uncertainty will be determined from results of NMIs/DIs that participate in the key comparison using IDMS method with demonstrated metrological traceability. However, other techniques may also be used for comparison

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[2] The Appendix may not contain an exhaustive list of all CRMs that may be used as calibration standards and quality control materials in this comparison.

purposes. Results from NMIs/DIs that participated in the pilot study will not be included in the calculation of the KCRV.

### Core Competency and How Far Does the Light Shine?

This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes in the range of 10 to 2,000 mg/kg, having the polarity $\log K_{OW} < -2$ and molecular mass of 50 to 500 Da in a biological matrix (such as human serum, blood and urine).

### Schedule

Official call for participation: 13 July 2015
Deadline for registration: 1 December 2015
Distribution of comparison samples: by 29 February 2016
Deadline for submission of results: 1 September 2016

### Coordinating Laboratory and Contact Person

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**Note:** A potential DI may also register to participate in the CCQM pilot study upon a written agreement from the NMI.

## Appendix

**CRMs of Urea**

| CRM Code | Source of CRM |
|---|---|
| SRM 912a (clinical standard) | NIST |
| CRM 6006-a | NMIJ |
| GBW09201 | NIM, China |

**CRMs of Uric Acid**

| CRM Code | Source of CRM |
|---|---|
| SRM 913a | NIST |
| CRM 6008-a | NMIJ |
| GBW09202 | NIM, China |

**Matrix CRMs Containing Urea and Uric Acid**

| CRM Code | Matrix Type | Source of CRM |
|---|---|---|
| SRM 909c | Frozen Human Serum | NIST |
| HRM-3002A | Frozen Human Serum | HSA |
| CRM 111-1-001 and CRM 111-01-002 | Frozen Human Serum | KRISS |
| CRM 111-1-003 and CRM 111-01-004 | Lyophilized Human Serum | KRISS |

**Matrix CRMs Containing Uric Acid**

| CRM Code | Matrix Type | Source of CRM |
|---|---|---|
| GBW09157 | Frozen Human Serum | NIM, China |
| GBW09169 | Frozen Human Serum | NIM, China |